Development of a Fathead Minnow Model for Evaluating Exposure of Fish to Genotoxic Substances

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Abstract

The fathead minnow (FHM) is widely used as a standard test species for acute and chronic toxicity testing of contaminants, effluents, and receiving waters. Because of its widespread distribution throughout North America, this species also has application in monitoring studies and for deployment using caged exposures in the field. However, the FHM has not been used previously as a tool for determining the genetic toxic effects of chemicals or complex environmental mixtures. In the present study, fathead minnows were exposed to varying dose levels of the known mutagen, methyl methanesulfonate, either by a single intraperitoneal injection or by static renewal in water. DNA damage was evaluated in liver and blood cells at 1 and 3 days after the start of exposure using the single cell gel electrophoresis (SCGE) assay. Dose-related increases in DNA damage were observed in both blood and liver using either exposure regimen. The maximum levels of damage in treated fish exceeded control levels by more than 10-fold. Studies are underway to examine DNA damage in FHM following acute and chronic exposures to the pesticide atrazine, both alone and in combination with other chemicals. These results indicate the feasibility of evaluating DNA damage in tissues of fathead minnows, and suggest that the SCGE assay in this species could be a useful addition to standard aquatic toxicity test protocols and an indicator method for assessing exposure to genotoxic agents in the field. This abstract has been subjected to Agency review and approved for publication.

Male and Female Fathead Minnows (*Pimephales promelas*) in a Breeding Chamber. *(Photo courtesy of Gary Ankley USEPA, Duluth, MN)*

Introduction

The fathead minnow (*Pimephales promelas*) plays a key role as an aquatic vertebrate toxicological model. This sexually dimorphic teleost has been used for decades as a sentinel for aquatic ecological risk assessment and remains a standard model for aquatic toxicity testing. The fish is also used extensively in a broad range of environmental analyses, from effluent monitoring to pesticide registration. Fathead minnow tests have generated acute life cycle, short-term chronic and embryo-larval toxicity information on numerous chemicals, municipal and industrial discharges and receiving waters. However, the fathead minnow has not been used previously for determining the genetic toxic effects of chemicals or complex environmental mixtures. The exposure of aquatic organisms to genotoxins has been associated with the development of tumors in individuals and with alterations in gene frequencies of populations. The objective of this study was to determine the value of the SCGE assay in the fathead minnow as an endpoint in standard aquatic toxicity tests and as an indicator of exposure to genotoxic agents.

Methods

Fish Culture and Maintenance:

- Adult fathead minnows (FHM), weighing between 1 and 8 grams, were obtained from laboratory cultures raised at the U.S. EPA facilities in Cincinnati, OH.
- Water quality (pH, temperature, dissolved oxygen, ammonia, nitrate and nitrite) was measured at the start and end of the exposure period. The water used during exposure was carbon filtered, deioinzed water supplemented with mineral salts to mimic tap water (referred to as synthetic, moderately hard, reconstituted water). Water was changed each day during the study.

Experimental Set-Up for Exposure of Fathead Minnows in Environmental Chambers

Exposure setup:

- FHM were exposed to test chemicals either by intraperitoneal injection or by static renewal in water. Exposure were carried out in 20 gal aquarium tanks for time course studies (three to five fish per treatment group) or in 1 gallon glass jars for two day duration in the static renewal exposures (three replicates with two fish/replicate). Temperature was controlled at 25˚C.
- Methyl methanesulfonate (MMS) was administered at doses of 0, 4 and 8 mg/kg by intraperitoneal injection or by static renewal in water at concentrations of 0.5 to 2 mg/L. Atrazine was administered by static renewal in water for two days at concentrations ranging from 0.05 to 5 mg/L.

SCGE Assay:

- Blood samples were drawn into heparinized syringes from caudal vein; the entire liver was dissected to prepare liver cell suspension.
- 100 cells per fish were scored using Komet image analysis software for the comet parameters tail length, % tail DNA, and tail moment (tail length x % tail DNA/100).
- Slides were prepared either by the conventional "agarose sandwich" slide procedure (Tice et al., Environ. Mol. Mutagen., 35, 206-221, 2000) or on Gelbond film using the method of McNamee et al. (Mutat. Res., 63-69, 2000) with minor modification.

Results

MMS Dose- and Time-Response (Figs. 1 and 2):

- Significant increases in DNA strand breaks were seen in liver and blood at 3 days after i.p. injection of 8 mg/kg MMS.
- Following static renewal exposure to MMS in water, significant dose-related increases in DNA damage were observed in blood within 24 hrs. By 3 days, a significant dose-response was also observed in liver and had increased substantially in blood. Overall, a significantly higher level of damage was induced in blood compared to liver.

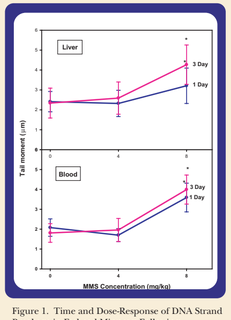

Figure 1. Time and Dose-Response of DNA Strand Breakage in Fathead Minnows Following Intraperitoneal Injection of MMS. * Indicates significantly increased over control at p≤0.1

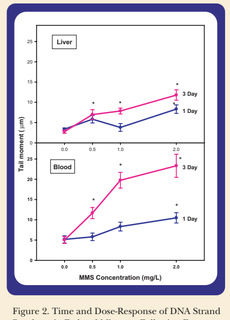

Figure 2. Time and Dose-Response of DNA Strand Breakage in Fathead Minnows Following Exposure to MMS in Water. * Indicates significantly increased over control at p≤0.05

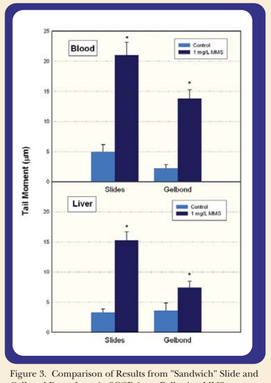

Figure 3. Comparison of Results from "Sandwich" Slide and Gelbond Procedures in SCGE Assay Following MMS Treatment of Fathead Minnows. * Indicates significantly increased over control at p≤0.05

Gelbond Modification of SCGE Assay (Fig. 3):

The level of damage induced by 1 mg/L MMS in blood cells was higher for the "sandwich" slide technique compared to the Gelbond procedure. However, the relative damage induced compared to control was higher for the Gelbond (6.1 fold) vs the slide method (4.1 fold). The Gelbond method appeared less sensitive for detecting induced damage in liver cells.

Assessment of Response to Atrazine (Fig. 4):

Atrazine did not induce DNA damage in blood cells of fathead minnows following exposure by static renewal of the compound in water for two days.

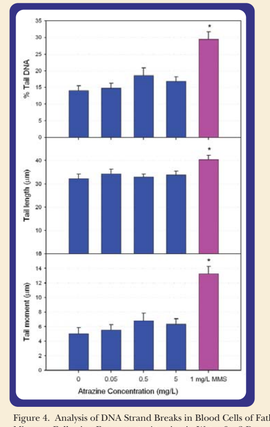

Figure 4. Analysis of DNA Strand Breaks in Blood Cells of Fathead Minnows Following Exposure to Atrazine in Water for 2 Days. * Indicates significantly increased over control at p≤0.05

Conclusions

- DNA damage was induced in a time- and dose-related manner in blood and liver of fathead minnows following exposure to methyl methanesulfonate, a known mutagen and carcinogen. DNA damage was not detected following exposure of fathead minnows to the pesticide atrazine.
- The Gelbond procedure was effective for detecting DNA damage in fathead minnows. This modification allows more rapid processing of samples in the SCGE assay. Improved sensitivity of the method may require optimization of alkaline unwinding and electrophoresis times.
- The results indicate that a DNA damage endpoint using the SCGE technique could be used in conjunction with standard aquatic toxicity tests with the fathead minnow. Genetic toxicity information would complement the well-developed knowledge base relating to methods and endpoints which already exists for this organism.

Future Work

- Investigate temporal DNA damage response following chronic exposure of fathead minnows to several known mutagens.
- Evaluate the suitability of using frozen tissues from fathead minnows in the SCGE assay.
- Investigate SCGE response of fathead minnows to environmentally relevant compounds and complex mixtures (e.g., effluents, sediments).
- Use differential display technique in conjunction with SCGE assay to discern differences in gene transcription following exposure of fathead minnows to various genotoxic chemicals.
- Identify up and down-regulated genes for subsequent development of single gene-transcription indicator methods for sensitive, rapid detection of genotoxin exposure.
- Investigate SCGE response and patterns of gene transcription in fathead minnows deployed in the field at contaminated vs reference locations.

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